PATENT COOPERATION TREATY



From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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# PCT

## NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

**(PCT Rule 71.1)**

| Date of Mailing *(day/month/year)* | 16 MAR 2005 |
|---|---|

| Applicant's or agent's file reference | IMPORTANT NOTIFICATION |
|---|---|
| 07917-166WO1 | |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US03/07323 | 07 March 2003 (07.03.2003) | 08 March 2002 (08.03.2002) |

Applicant

UNIVERSITY OF MASSACHUSETTS

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

| Name and mailing address of the IPEA/US | Authorized officer |
|---|---|
| Mail Stop PCT, Attn: IPEA/US<br>Commissioner for Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450 | Zachariah Lucas |
| Facsimile No. (703) 305-3230 | Telephone No. 571-272-1600 |

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

**(PCT Article 36 and Rule 70)**

| Applicant's or agent's file reference<br>07917-166WO1 | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/US03/07323 | International filing date *(day/month/year)*<br>07 March 2003 (07.03.2003) | Priority date *(day/month/year)*<br>08 March 2002 (08.03.2002) |
| International Patent Classification (IPC) or national classification and IPC<br>IPC(7): C12N 7/01 and US Cl.: 435/235.1 | | |
| Applicant<br>UNIVERSITY OF MASSACHUSETTS | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 13 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 08 October 2003 (08.10.2003) | 18 February 2005 (18.02.2005) |
| Name and mailing address of the IPEA/US<br>Mail Stop PCT, Attn: IPEA/US<br>Commissioner for Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450<br>Facsimile No. (703) 305-3230 | Authorized officer<br>Zacharian Lucas<br>Telephone No. 571-272-1600 |

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/07323

## I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed.

☒ the description:
pages 1-4, 6, 8-16, 19-23, 25-29 as originally filed
pages 5,7,17-18,24, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages 30-33, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the drawings:
pages NONE, as originally filed
pages 1-8, filed with the demand
pages NONE, filed with the letter of _____.

☐ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages NONE

☐ the claims, Nos. NONE

☐ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/07323

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The question whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 7-23

because:

☐ the said international application, or the said claim Nos. ____ relate to the following subject matter which does not require international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. ____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. ____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 7-23

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/07323

**V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. STATEMENT

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 4-6,25,28 and 30-34 | YES |
| | Claims | 1-3,24,26,27 and 29 | NO |
| Inventive Step (IS) | Claims | 5 | YES |
| | Claims | 1-4,6 and 24-34 | NO |
| Industrial Applicability (IA) | Claims | 1-6 and 24-34 | YES |
| | Claims | NONE | NO |

2. CITATIONS AND EXPLANATIONS

Claims 1-3, 24, 26, 27, and 29 lack novelty under PCT Article 33(2) as being anticipated by U.S. Patent 5,985,655 (Anderson et al.). These claims describe virus particles with chimeric envelope proteins, and methods of using such particles to deliver nucleic acids to a cell. Such particles and methods are disclosed by Anderson. Abstract, columns 1-2, and 5-6. The reference therefore anticipates the indicated claims. In view of the reference, the claims lack novelty.

Claims 1-4, 6, 24-27, and 29 lack an inventive step under PCT Article 33(3) as being obvious over U.S. Patent 5,736,387 (Paul et al.). These claims have been described in part above, except that claims 4 and 25 describe embodiments wherein the ligand inserted in to the chimeric envelope protein is a ligand for the epidermal growth factor receptor. Such viral particles are suggested by the Paul patent. See, columns 1-4 (esp. column 4), and column 9 (suggesting the use of the EGF cytokine as the cytokine ligand). The reference therefore demonstrates that the claims lack an inventive step over the art.

Claims 28 and 30-34 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the preceding paragraphs and further in view of FERNANDEZ et al., and SCHNIERLE et al. These claims are directed to methods wherein the viral particles are used to deliver nucleic acids to cancer cells, or to treat a cancer. The teachings of Anderson and Paul as described above suggest the use of the claimed viral particles to deliver nucleic acids to specific cells. The teachings of Fernandez and Schnierle suggest the use of viral particles comprising thymidine kinase genes, or viral particles with chimeric envelope proteins, for the treatment of malignant disorders. From these combined teachings, it would have been obvious to those in the art to use virus particles comprising the chimeric envelope proteins to deliver nucleic acids, including those encoding thymidine kinase, to cancer cells. The claims thus lack an inventive step over the prior art.

Fig. 2 is a bar graph showing the results of experiments testing the ability of $RGD_{21}$ viruses to transduce NIH 3T3 cells and A375 human melanoma cells.

Figs. 3A-3B are bar graphs illustrating transduction experiments testing the requirement of the RGD sequence for transduction of human cells. (A) Transduction of NIH 3T3 infected with an $RGD_{21}$ or $RGE_{21}$ virus, and (B) Transduction of A375 human melanoma cells infected with an $RGD_{21}$ or $RGE_{21}$ virus.

Figs. 4A-4B are bar graphs showing the results of experiments testing the effect of pretreatment with antibodies to integrin receptors on transduction of human cells by RGD viruses (A) NIH 3T3 cells; (B) A375 human melanoma cells.

Fig. 5 is a bar graph showing the results of experiments testing the ability of GRP viruses to transduce human cells.

Figs. 6A-6C are bar graphs showing the results of experiments examining the requirement of the GRP receptor for transduction of human cells by GRP viruses. (A) Antibodies to GRP block transduction of human cells by GRP viruses. (B) Requirement of the GRP receptor for transduction of human 293 cells. (C) Requirement of the GRP receptor for transduction of mouse cells by GRP-2, GRP-3 and GRP-5 viruses.

Figs. 7A-7B are bar graphs showing the results of experiments testing the ability of HRG viruses to transduce NIH 3T3 cells and MDA-MB-453 breast carcinoma cells. (A) Transduction of NIH 3T3 cells by HGR viruses. (B) Transduction by HRG-1 or HRG-8 virus after pretreatment of NIH 3T3 and MDA-MB-453 breast carcinoma cells with antibodies to HER-3 and HER-4 receptors.

Fig. 8 is a representation of the nucleic acid sequence of MoMLV envelope protein.

## DETAILED DESCRIPTION

The invention provides a strategy for altering the host range of ecotropic retrovirus vectors using a recombinant envelope protein that contains a heterologous short peptide ligand (chimeric envelope proteins). Viruses expressing such chimeric envelope proteins (pseudotyped virus) can transduce human cells without removal of the N-terminal region of the naturally occurring envelope protein or co-expression of wild-type envelope protein. Furthermore, it is not necessary to delete portions of the

display in which a library of phage bearing a random selection of small peptides is selected for binding to the extracellular domain of a cell surface protein (i.e., a cell surface protein expressed on a host target cell). Nucleic acid sequences coding for such peptides are then cloned into wild-type envelope protein to produce chimeric envelope proteins. In another method using phage library, targeting to various organs can be achieved by injecting a phage display library into animals and identifying the peptides localized in each organ. This method has been successfully used to identify short peptides targeted to, e.g., kidney cells (CLPVASC, SEQ ID NO:3; CLPVASC, SEQ ID NO:4; and CGAREMC, SEQ ID NO:5) and to brain cells (CLSSRLDAC, SEQ ID NO:6; WRCVLREGPAGGCAWFNRHRL; SEQ ID NO:7) (Pasqualini et al., 1996, Nature 380:364-366). Similarly, recombinant peptide libraries can also be screened for peptides that specifically bind to a protein that is expressed on a target host cell (Pasqualini *supra*; Wrighton et al., 1996, Science 273:458-464; Cwirla et al., 1997, Science 276:1696-1699; Arap et al., 1998, Science 279:377-380).

*Chimeric Envelope Proteins and Libraries*

Envelope proteins are known in the art. In particular, the ecotropic murine leukemia virus protein has been extensively studied. The sequence of the MoMLV envelope protein (gp70) is shown in Fig. 8. The sequence coding for the extracellular domain (SU) region of the envelope protein extends from nucleotides 5612–6919. The transmembrane region and cytoplasmic tail extend from nucleotides 6920-7507. There is a signal peptide sequence at the beginning of the SU, that localizes the protein to the cell membrane. Clones containing MoMLV envelope protein are commercially available (e.g., Stratagene, La Jolla, CA). Heterologous short peptide ligands are inserted in the extracellular domain of the envelope protein. In general, chimeric envelope proteins containing insertions near the N-terminus and in the proline-rich region (PRR region) of the envelope protein are less effective for altering viral tropism than insertions at other positions within the protein. Examples of specific insertion locations that are effective are described herein, and in detail in the Examples.

Transduction efficiency also depends on the presentation of the ligand within the envelope. In some embodiments of the invention, cysteine residues flank the

Table 1. Description of RGD viruses.

| ENV # | Position of Ligand Insertion (A.A. Location) | # of Inserts | Deletion of Nucleotides in Env. |
|---|---|---|---|
| **$RGD_{13}$** [CAAA- GRGDSP-TRC] | | | |
| 1 | 1 | 1X | |
| 2 | 1 | 2X | |
| 3 | 1 | 4X | |
| 4 | 38 | 1X | |
| 5 | 38 | 3X | |
| 6 | 38 | 1X | 5990-6082 |
| 7 | 68 | 1X | |
| 8 | 68 | 2X | |
| 9 | 68 | 1X | 6082-6191 |
| 10 | 120 | 1X | |
| 11 | 120 | 2X | 6238-6281 |
| 12 | 120 | 3X | |
| 13 | 185 | 1X | |
| 14 | 230 | 1X | |
| 15 | 230 | 2X | |
| 16 | 235 | 1X | |
| 17 | 235 | 4X | |
| 18 | 310 | 1X | |
| 19 | 310 | 2X | |
| 20 | 321 | 1X | |
| 21 | 321 | 2X | |
| 22 | 382 | 1X | |
| 23 | 382 | 2X | |
| 24 | 382 | 3X | |
| 25 | 388 | 1X | |
| 26 | 388 | 2X | |
| **$RGD_{21}$** [CAAA- QGATFALRGDNPQG-TRC] | | | |
| 1 | 1 | 1X | |
| 2 | 38 | 1X | |
| 3 | 38 | 1X | 5990-6082 |
| 4 | 68 | 1X | |
| 5 | 68 | 1X | 6082-6191 |
| 6 | 120 | 1X | |
| R | 120 | 1X | 6238-6281 |

| | | | |
|---|---|---|---|
| 8 | 185 | 1X | |
| 9 | 230 | 1X | |
| 10 | 235 | 1X | |
| 11 | 310 | 1X | |
| 12 | 321 | 1X | |
| 13 | 382 | 1X | |
| 14 | 388 | 1X | |
| 15 | 1,68 | 1X,1X | |
| 16 | 1,230 | 1X,1X | |

**$RGE_{21}$** [CAAA- QGATFALRGENPQG-TRC]

| | | | |
|---|---|---|---|
| 1 | 1 | 1X | |
| 2 | 38 | 1X | 5990-6082 |
| 3 | 68 | 1X | |
| 4 | 68 | 1X | 6082-1916 |
| 5 | 230 | 1X | |

The core of the $RGD_{13}$ ligand is a six amino acid peptide, GRGDSP (SEQ ID NO:14), which represents an RGD consensus sequence. The core of the $RGD_{21}$ ligand is a 14 amino acid sequence, QGATFALRGDNPQG (SEQ ID NO:15), derived from the mouse laminin protein (Aumailley et al., 1990, FEBS Lett. 262:82-86). Both the $RGD_{13}$ and $RGD_{21}$ peptides were flanked by cysteine residues to constrain the sequence within a loop (Aumailley et al., 1990, *supra*; Yamada et al., 1993, J. Biol. Chem. 268:10588-10592; Hart et al., 1994, J. Biol. Chem. 269:12468-12474; Pierschbacher and Ruoslahti, 1987, J. Biol. Chem. 262:17294-17298).

In some cases, chimeric envelope proteins with multiple ligands in tandem were also generated. Several of the chimeric envelope proteins had deletions of envelope sequences, in addition to ligand insertions, as a result of multiple restriction enzyme cleavages. In total, 26 chimeric envelope proteins containing the $RGD_{13}$ ligand, 16 chimeric envelope proteins containing the $RGD_{21}$ ligand, and five chimeric envelope proteins containing an $RGE_{21}$ ligand, a control non-binding peptide (Aumailley et al., 1990, *supra*; Hart et al., 1994, *supra*; Solowska et al., 1989, J. Cell Biol. 109:853-861; Greenspoon et al., 1993, Biochemistry 32:1001-1008), were constructed.

Table 2. Description of GRP and HRG viruses

| ENV # | Position of Ligand Insertion (A.A. Location) | Deletion of Nucleotides in Envelope |
|---|---|---|
| GRP CAAA – **EQRLGNQWAVGHLM** – TRC | | |
| GRP-1 | 1 | |
| GRP-2 | 38 | |
| GRP-3 | 38 | 5990-6082 |
| GRP-4 | 68 | |
| GRP-5 | 68 | 6082-1916 |
| GRP-6 | 120 | |
| GRP-7 | 120 | 6238-6281 |
| GRP-8 | 185 | |
| GRP-9 | 230 | |
| GRP-10 | 235 | |
| GRP-11 | 310 | |
| GRP-12 | 321 | |
| GRP-13 | 382 | |
| GRP-14 | 388 | |

Del. 3 A.A.
|
FM D PSRY L M

HRG CAAA –
**SHLVKCAEKEKTFCVNGGECYRVKTYGYLMCKCPNEFTGDRCQNYVIAS** – TRC

| | | |
|---|---|---|
| HRG-1 | 1 | |
| HRG-2 | 38 | |
| HRG-3 | 38 | 5990-6082 |
| HRG-4 | 68 | |
| HRG-5 | 68 | 6082-1916 |
| HRG-6 | 120 | |
| HRG-7 | 185 | |

Rec'd PCT/PTO 08 SEP 2004

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REPLACED BY
ART 34 AMDT

Figure 1

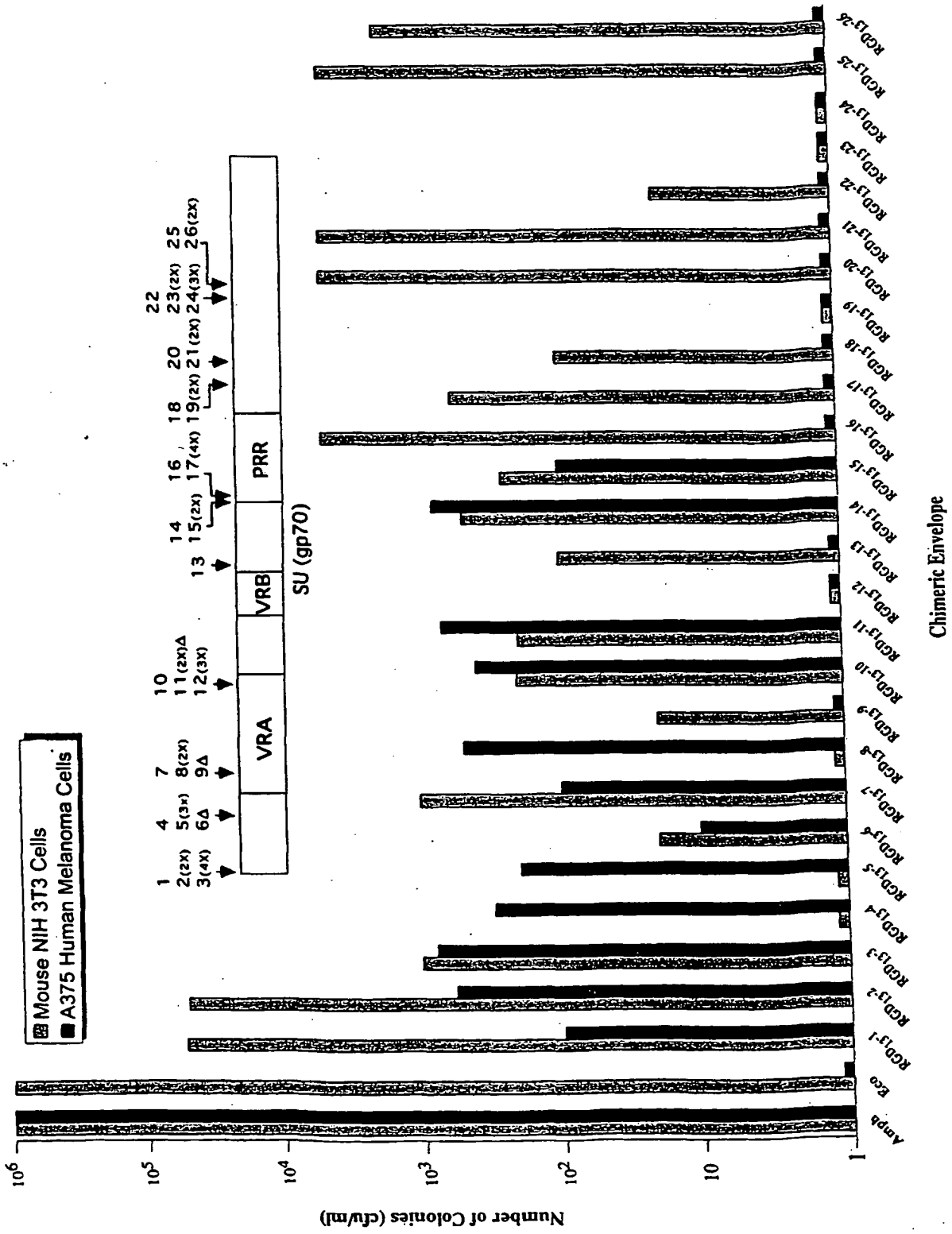
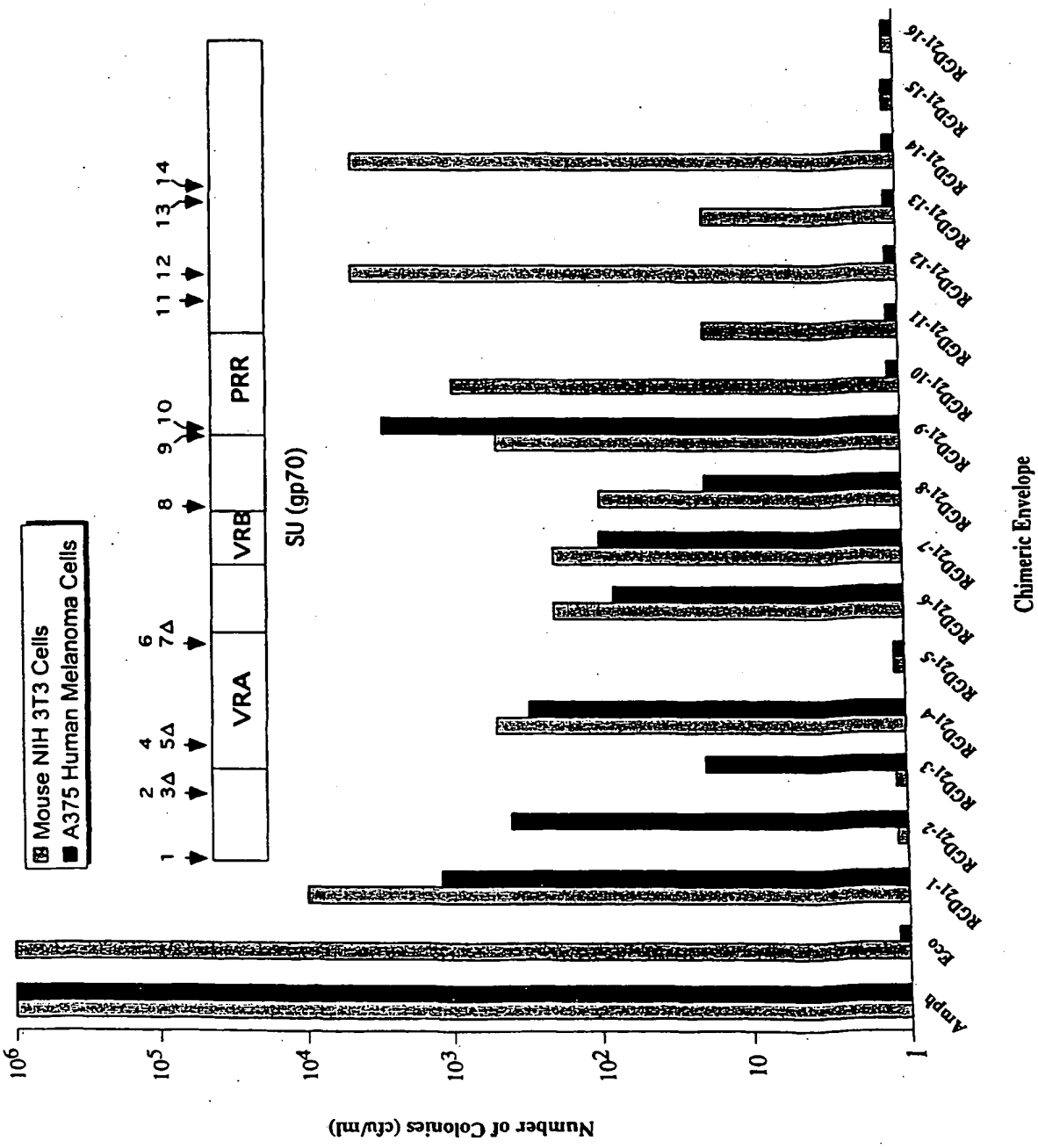


Figure 2



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ART 34 AMDT

Figure 3

Fig. 3B

A375 Human Melanoma Cells

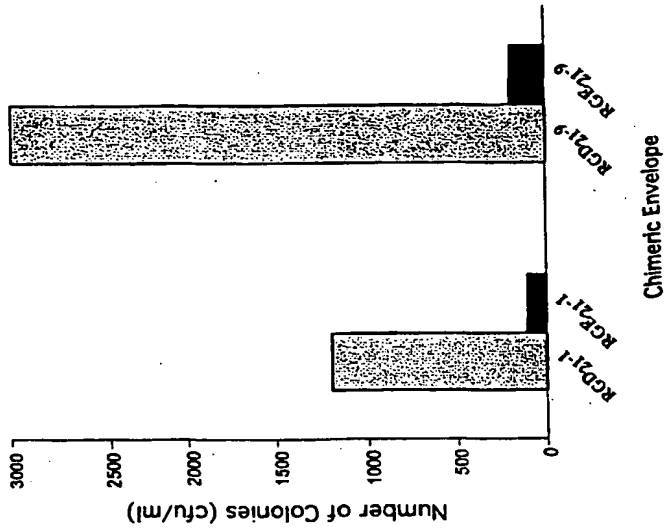


Fig. 3A

Mouse NIH 3T3 Cells

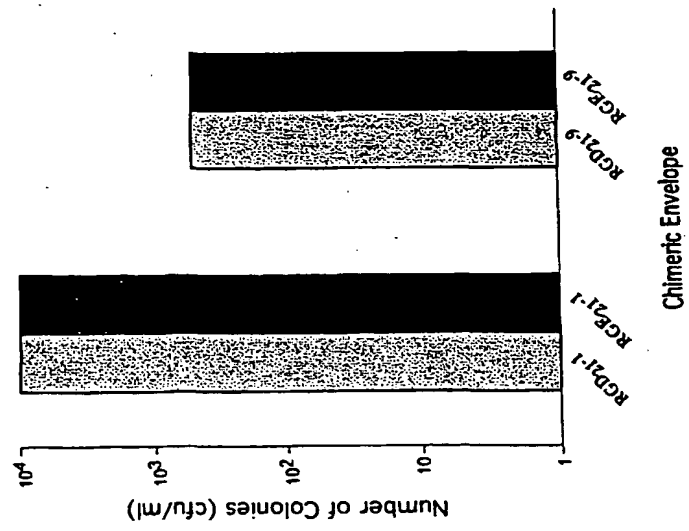

Figure 4

Fig. 4B

A375 Human Melanoma Cells

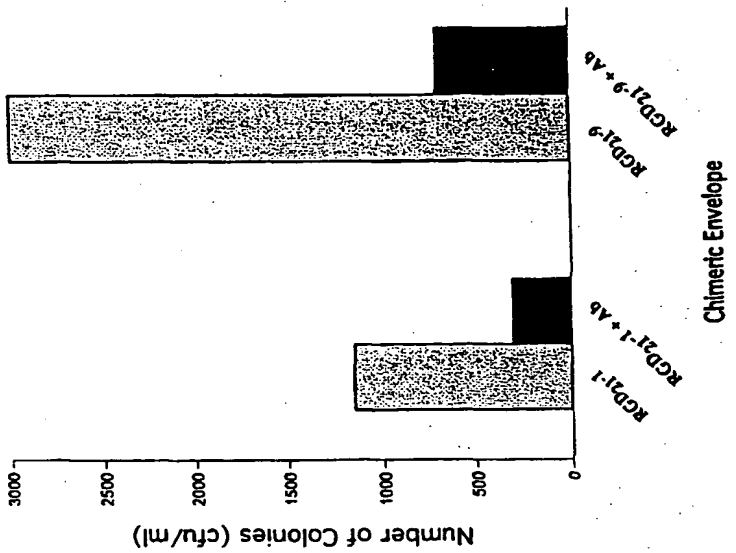


Fig. 4A

Mouse NIH 3T3 Cells

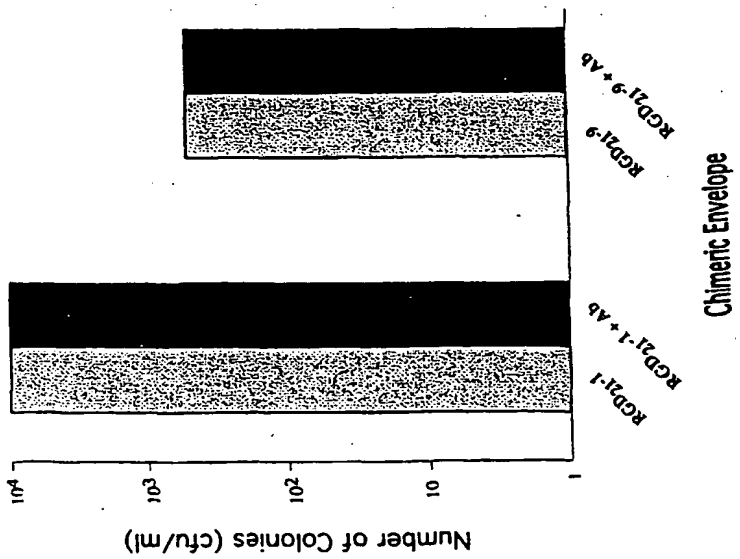

Figure 5

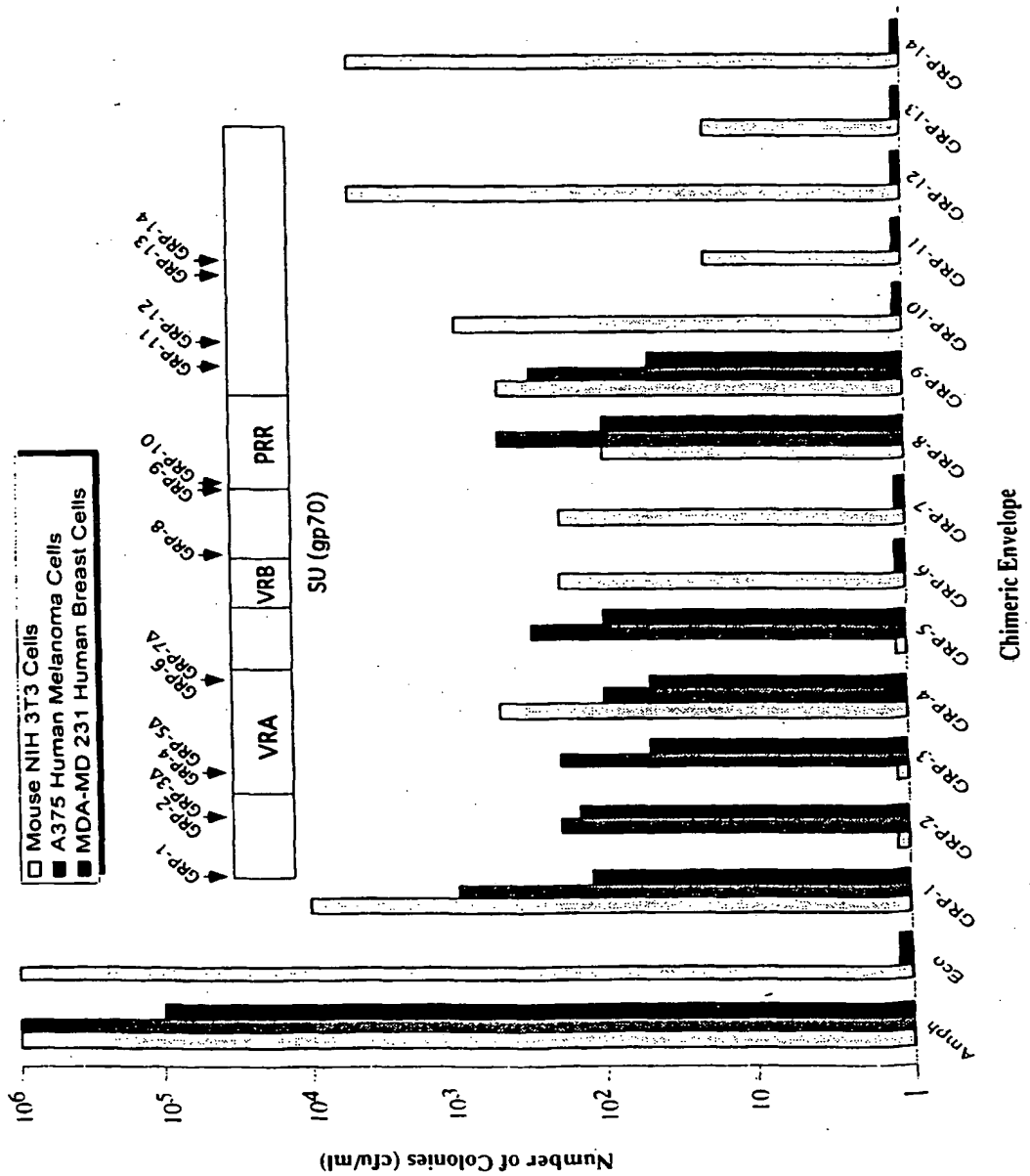

Figure 6

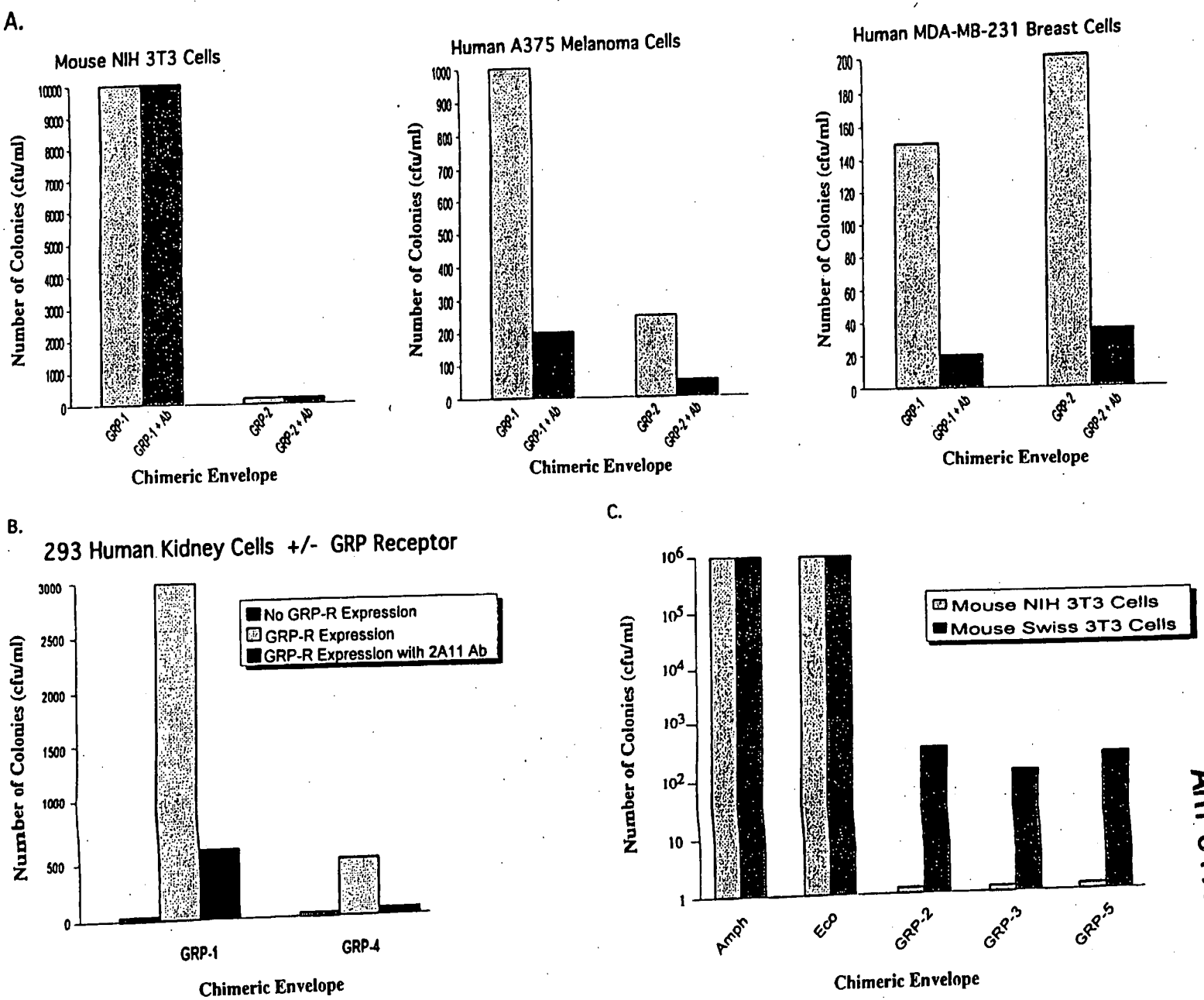

Figure 7

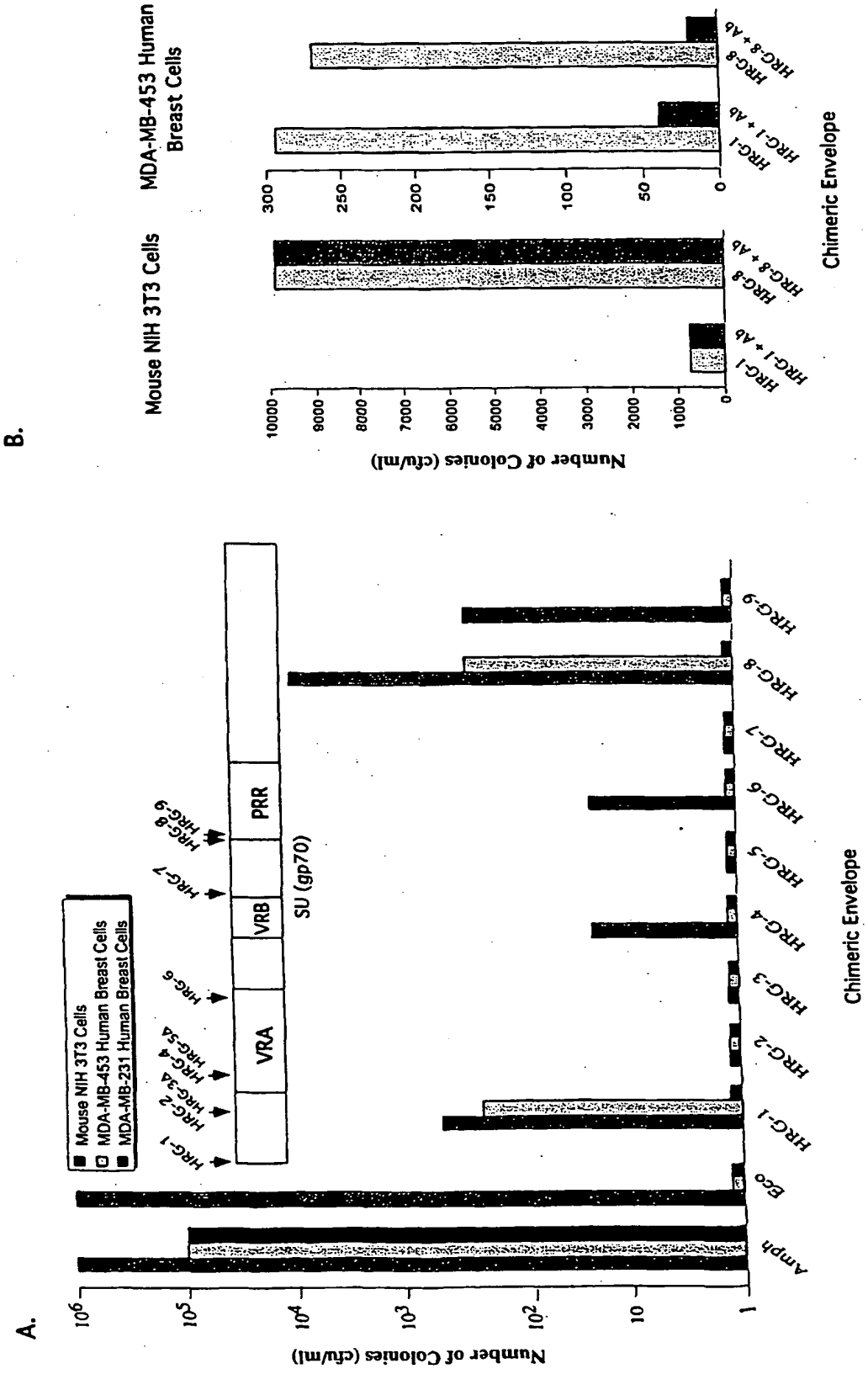

Fig. 8

Moloney Murine Leukemia Virus – envelope protein (gp70), nucleic acid sequence (from complete MoMLV genome sequence; Genbank Accession No. NC_001501). The SU (extracellular domain) is coded by nucleotides 5612 – 6919 (pictured below). The transmembrane and cytoplasmic tail extends from nucleotides 6920-7507. There is a signal peptide sequence at the beginning of the SU, localizing the protein to the cell membrane.



5581 aattcttctg atgctcagag gggtcagtac tgcttcgccc ggctccagtc ctcatcaagt
5641 ctataatatc acctgggagg taaccaatgg agatcgggag acggtatggg caacttctgg
5701 caaccaccct ctgtggacct ggtggcctga ccttacccca gatttatgta tgttagccca
5761 ccatggacca tcttattggg ggctagaata tcaatcccct ttttcttctc ccccggggcc
5821 cccttgttgc tcagggggca gcagcccagg ctgttccaga gactgcgaag aacctttaac
5881 ctccctcacc cctcggtgca acactgcctg gaacagactc aagctagacc agacaactca
5941 taaatcaaat gagggatttt atgtttgccc cgggccccac cgcccccgag aatccaagtc
6001 atgtgggggt ccagactcct tctactgtgc ctattggggc tgtgagacaa ccggtagagc
6061 ttactggaag ccctcctcat catgggattt catcacagta aacaacaatc tcacctctga
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6181 agacgccggg agacgggtta cttcctggac cacaggacat tactggggct tacgtttgta
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6301 accccgcgtc ccaatagggc caaacccccgt tctggcagac caacagccac tctccaagcc
6361 caaacctgtt aagtcgcctt cagtcaccaa accacccagt gggactcctc tctcccctac
6421 ccaacttcca ccggcgggaa cggaaaatag gctgctaaac ttagtagacg gagcctacca
6481 agccctcaac ctcaccagtc ctgacaaaac ccaagagtgc tggttgtgtc tagtagcggg
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6601 agccaactgc tccgtggcct cccaacacaa gttgaccctg tccgaagtga ccggacaggg
6661 actctgcata ggagcagttc ccaaaacaca tcaggcccta tgtaatacca cccagacaag
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6961 aaccatgggg ggaattgccg ctggaatagg aacagggact actgctctaa tggccactca
7021 gcaattccag cagctccaag ccgcagtaca ggatgatctc agggaggttg aaaaatcaat
7081 ctctaaccta gaaaagtctc tcacttccct gtctgaagtt gtcctacaga atcgaagggg
7141 cctagacttg ttatttctaa aagaaggagg gctgtgtgct gctctaaaag aagaatgttg
7201 cttctatgcg gaccacacag gactagtgag agacagcatg gccaaattga gagagaggct
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